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Effects of pressure differentials on the viability and infectivity of entomopathogenic nematodes☆

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Abstract

During passage through the different components of a spray application system, a nematode suspension will undergo pressure changes. The extent of damage to three species of entomopathogenic nematodes (EPNs) (*Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, and *Heterorhabditis megidis*) in suspension due to the effects of a pressure differential was studied. A French pressure cell and press was used to subject the newly emerged EPN suspensions to a series of pressure differentials ranging from 1283 kPa (186 psi) to 10,690 kPa (1550 psi). Aged suspensions (3 weeks) of *H. bacteriophora* and *H. megidis* were also evaluated. Damage was quantified by counting living and dead (whole and pieces) EPNs and by bioassay techniques. As the pressure differential increased, the relative viability of the EPNs decreased. Entomopathogenic nematodes that survived the pressure differential were, in general, able to survive for at least 1 week and maintain infectivity to *Galleria mellonella* at rates equivalent to EPNs that had not been pressure treated. In general, the relative viabilities of fresh and aged EPNs were equivalent after pressure differential treatments. The relative viability of the treated EPNs remained above 85% for pressure differentials less than or equal to 1283 kPa for *H. megidis* and 2138 kPa (310 psi) for *S. carpocapsae* and *H. bacteriophora*, but decreased rapidly for higher pressure differentials. Greater reductions in relative viability were experienced by *Heterorhabditis* spp. than *S. carpocapsae*, indicating that nematode species is an important factor to consider when defining spray operating conditions. We recommend a maximum operating pressure of 1380 kPa (200 psi) for *H. megidis* and 2000 kPa (290 psi) for *S. carpocapsae* and *H. bacteriophora*.

Keywords: *Steinernema carpocapsae*; *Heterorhabditis bacteriophora*; *Heterorhabditis megidis*; *Galleria mellonella*; Age; Damage; Application; Spray equipment

1. Introduction

Entomopathogenic nematodes (EPNs) have been recognized as excellent biological control agents of soil-dwelling insect pests (Klein, 1990). Recent advances in mass-production and formulation technology have made insecticidal EPNs available commercially for large-scale application in citrus groves, strawberry plantations, cranberry bogs, artichokes, mint, mushrooms, ornamentals, and turfgrass (Grewal and Georgis, 1998). Field efficacy studies have shown that EPNs in the genera *Steinernema* and *Heterorhabditis* can be effective biological control agents against a wide variety of soil insect pests and for various cropping systems, such as black vine weevil (*Otiorhynchus sulcatus* F.) in cranberry bogs (Hayes et al., 1999) and strawberry fields (Curran, 1992), citrus root weevils (*Diaprepes abbreviatus* L. and *Pachnaeus litus* Germar) in citrus groves (Bullock et al., 1999), or the alfalfa snout beetle (*Otiorhynchus ligustici* L.) in alfalfa fields (Shields et al.,

☆ Product and company names are necessary to report factually on available data; however, the Ohio State University and USDA neither guarantee nor warrant the standard of the product, and the use of the name by OSU or USDA implies no approval of the product to the exclusion of others that may also be suitable.

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1999). Despite these successes, insecticidal EPNs are not yet reducing reliance on chemical insecticides to any significant degree (Grewal and Georgis, 1998).

One area of research that has received little attention with respect to all biological control agents is application technology (Mason et al., 1999). In practice, it has generally been considered that EPNs can be applied with conventional agricultural spray equipment (Georgis, 1990). The general recommendation for EPN application has been common nozzle type sprayers with openings larger than 50 μm and operating pressures less than 2000 kPa (Georgis, 1990; Shetlar, 1999). However, no studies were cited to support these recommendations, which are most likely based on information from *Steinernema carpocapsae* (Weiser), the most widely studied and available EPN, and may not be representative for all EPN species.

Spray equipment can vary considerably from tractor-drawn boom sprayers used in field crops to backpack sprayers used in greenhouses. Generally, a liquid application system consists of a holding container, pump, valve(s), spray line, and nozzle(s). The EPNs will settle out of suspension within a short period of time, so there must be agitation in the holding container either through re-circulation of a portion of the spray mix or mechanical stirring. Pumps and valves are needed for liquid movement and flow control and may damage the EPNs through producing high shear forces or enough heat to be detrimental. Also, with hydraulic atomization, the liquid suspension is forced through an orifice under high pressure (generally less than 2000 kPa), which may damage the EPNs.

Previous studies have indicated that application technique has a significant effect on efficacy of the EPNs in field and greenhouse trials (Curran, 1992; Hayes et al., 1999; Piggott, 2000; Shields et al., 1999). In general, these studies focused on EPN placement and distribution when comparing application methods and efficacy. While dispersal information is important, differences in the application equipment components and their potential to damage EPNs has been largely ignored, with a few exceptions (Hayes et al., 1999; Klein and Georgis, 1994; Nilsson and Gripwall, 1999). Hayes et al. (1999) found about 95% mortality of *Galleria mellonella* (L.) by *S. carpocapsae* using a multiple nematode infectivity assay after the nematodes were passed through a sprinkler irrigation system and a boom sprayer. *G. mellonella* larvae are extremely susceptible to nematode infection (Dutky et al., 1962), and multiple nematode assays (Woodring and Kaya, 1988) have been considered inappropriate to measure the proportion of a population which is infective (Converse and Miller, 1999; Grewal et al., 1999). Thus, evidence indicates that the infectivity results of Hayes et al. (1999) do not provide adequate information with respect to the effects of application equipment on EPN damage. Nilsson and Gripwall (1999) found no significant reduction in viability of *Steinernema feltiae* (Filipjev) after spraying with a backpack sprayer (200 kPa, diaphragm pump, Hardi 4110-12 fan nozzle) and a high-pressure sprayer (1000 and 2000 kPa, piston pump, 1.2 mm Wanjet pressure swirl solid cone). However, they noted a tendency of reduced viability of the nematodes in all the high-pressure sprayer treatments, and a significant decrease in viability as the length of the pumping period in the high-pressure sprayer increased. Klein and Georgis (1994) reported that no adverse effects were observed for *Steinernema* spp. and *Heterorhabditis bacteriophora* (Poinar) after flow through several different pumps (piston, centrifugal, roller, and diaphragm), nozzle types (Spraying Systems XR8001VS, TK-VS2, FL-5VS), and strainers (100 mesh, 50 mesh, 50 slotted). However, Klein and Georgis (1994) did not report any data explicitly.

Therefore, at present, there are few research-based guidelines on how EPNs should be applied to optimize their performance in the field. One approach is to evaluate the different physical phenomenon occurring within an application system. During passage through a spray application system, the liquid suspension will undergo significant pressure changes. In particular, a large pressure drop occurs as the suspension moves through the nozzle exit orifice from the system operating pressure to the atmosphere. Rapid changes in pressure may cause the nematode cuticle to break or burst, thus causing permanent damage and reducing the potential efficacy.

The objective of this study was to evaluate the effect of pressure differentials on the survival and infectivity of a benchmark biological pest control agent, the entomopathogenic nematode, and to determine whether EPN species and age are important factors.

2. Materials and methods

2.1. Entomopathogenic nematodes

Three species of EPNs were studied: *S. carpocapsae* All strain, *H. bacteriophora* GPS 11 strain, and *Heterorhabditis megidis* (Poinar, Jackson and Klein) UK strain. The EPNs were cultured in vivo in the laboratory using last-instar *G. mellonella* (Vanderhorst Canning, St. Mary's, OH) as the host and standard culture procedures (Kaya and Stock, 1997). The harvested EPN suspensions were stored in 150 × 20-mm petri dishes at 5 °C for *S. carpocapsae* and 10 °C for *H. bacteriophora* and *H. megidis* until tests were conducted within a week following harvest. These EPNs were classified as freshly reared.

A separate test was conducted on aged *H. bacteriophora* and *H. megidis*. The fresh EPN suspensions were

diluted to approximately 1000 EPNs/ml, and 50 ml of the suspensions were placed in several 150 × 20-mm petri dishes for storage in a 25 °C chamber, allowing for sufficient oxygen exchange. The suspensions were stored for 3 weeks before being tested. Distilled water was added to the dishes as needed.

2.2. Effect of a pressure differential

The French pressure cell and press (Spectronic Unicam, Rochester, NY) was used to determine the effect of various pressure differentials on EPNs in suspension. The press is a hydraulic press that uses control valves and a motor-driven pump to vary hydraulic pressure generated by the press. A 10-ml nematode suspension sample, thoroughly mixed, was poured into the French pressure cell chamber. All of the air in the cell chamber was removed by manually pushing the piston into the cell chamber until fluid escaped from the sample outlet orifice. About 4–5 ml was expelled from the chamber, leaving about 5–6 ml of suspension in the chamber for testing. The exit orifice is a 52-mm steel tube, 1.5 mm in diameter that extends out of the closure plug on the chamber assembly. The piston and chamber assembly was placed on the French press stand and clamped into place, and the flow release valve was secured. The pressure increase control dial was turned to the desired press gauge pressure. The pressure inside the cell chamber was increased from atmospheric pressure to the desired pressure as the piston was pushed into the cell chamber by the press. The suspension was then released through the sample outlet orifice one droplet at a time (approximately one droplet every 5 s) by opening the steel needle valve until no more droplets exited the orifice. The treated sample was collected in a 55 × 10-mm petri dish and saved for observation. Ten pressure differential treatments were administered to each nematode species: 0 kPa (control), 1283 kPa (186 psi), 1710 kPa (248 psi), 2138 kPa (310 psi), 2566 kPa (372 psi), 2993 kPa (434 psi), 3421 kPa (496 psi), 3848 kPa (558 psi), 4276 kPa (620 psi), 6414 kPa (930 psi), and 10,690 kPa (1550 psi). The control sample was collected from the suspension that was expelled when bleeding air out of the chamber. For the fresh nematodes, there were 3 replicates per treatment for *S. carpocapsae* and 2 replicates per treatment for *H. bacteriophora* and *H. megidis*. For the aged nematodes, there was 1 replicate per treatment.

2.3. Effect of a static pressure

The French pressure cell and press was used to determine the effect of a static pressure on EPNs in suspension that were exposed to a pressure of 3421 kPa for 0 (control), 5, 10, and 30 min. A 10-ml sample of *H. bacteriophora* suspension was placed in the cell chamber. The same procedures were followed as before, except that the sample was not released from the sample outlet orifice. Rather, after the designated time period, the chamber was depressurized back to atmospheric pressure. The initial suspension concentration was considered the control.

2.4. Viability

Nematodes were counted by collecting a 10-μl subsample with a micro-dispenser from a thoroughly mixed suspension, and adding approximately 10 ml of water in a 55 × 10-mm petri dish to allow for easy viewing with the microscope. Three subsamples per replication were counted for all treatments. Nematode viability was determined by separately recording the number of live, dead, half pieces, and quarter pieces of nematodes. Nematodes were considered dead if they were broken or did not respond to prodding.

The total number of dead EPNs per subsample (TD) was computed as follows

$$\mathrm{TD} = \sum \left(D + \frac{\mathrm{HP}}{2} + \frac{\mathrm{QP}}{4} \right), \tag{1}$$

where D is the number of dead whole EPNs, HP is the number of half pieces of EPNs, and QP is the number of quarter pieces of EPNs. Relative viability of the EPNs immediately after treatment (RV, %) was computed as follows

$$\mathrm{RV} = \frac{L}{L + \mathrm{TD}} \times 100, \tag{2}$$

where L is the number of living EPNs. Relative viability of the EPNs after survival for 1 week at room temperature (SRV, %) was computed as follows.

$$\mathrm{SRV} = \frac{L}{L + \mathrm{D}} \times 100. \tag{3}$$

Only living and dead whole EPNs were considered in Eq. (3) (i.e., no nematode pieces) in order to determine if the pressure treatments had any effect on the ability of living EPNs to survive for at least 1 week. When comparing the survival after 1 week to the same day as the pressure treatment, the relative viability of the EPNs from the same day as the pressure treatment were also computed using Eq. (3).

2.5. Infectivity

Entomopathogenic nematode infectivity to *G. mellonella* larvae was determined using the sand-well bioassay (Grewal et al., 1999). Briefly, either one (i.e., *S. carpocapsae*) or five (i.e., *H. bacteriophora* and *H. megidis*) randomly selected living EPN(s) were transferred using a micro-dispenser along with 108 μl water into a single plate well (24-well tissue culture plate with 15.6-mm-diameter wells) containing 1.8 g of play sand (Play Sand,

Quikrete Companies, Atlanta, GA). An individual *G. mellonella* larva was added to the top of each well and the plate was sealed with parafilm to minimize moisture loss. One 24-well plate was prepared for each treatment replication. All plates were incubated at room temperature, and the number of dead *G. mellonella* larvae were counted per plate after 72 h. For each replication, a plate with water but no EPNs was included as a control for *G. mellonella*. The average percent infectivity of EPNs against *G. mellonella* for each treatment was determined by taking the average number of dead *G. mellonella* larvae for the treatment, subtracting the average number of dead *G. mellonella* larvae for the control, and dividing by 24 (the number of wells per plate). In addition, a one-on-one bioassay was conducted with *H. megidis* because little is known about the infection rates of this nematode.

2.6. Statistical analyses

Data on relative viability of fresh EPNs were arcsine transformed and analyzed by a completely randomized, 3×11 factorial ANOVA with subsampling, and EPN species and pressure differential treatment effects. The treatment and total sums of squares were determined using the PROC GLM procedure in SAS (SAS, 1994). The subsampling sum of squares was determined by computing the sum of squares for each set of subsamples and then summing these values. The error sum of squares was computed from the residual. Least significant differences (LSD) were used to compare individual treatment means at a significance level of 0.05.

Comparisons of EPN survival after 1 week to directly after treatment at each pressure differential level (for each EPN species) and for fresh and aged EPNs at each pressure differential level (for *H. bacteriophora* and *H. megidis*) was performed using Student's *t* tests. Data on EPN infectivity were analyzed for each EPN species using regression analysis to determine if EPN infectivity varied as a function of pressure differential.

3. Results and discussion

3.1. Effect of pressure differentials on fresh EPNs

The French pressure cell was used in this study because it provided a controlled environment for determining the effect of pressure differentials on EPNs, effectively removing any variables due to pumping or atomization that would have been present if actual spray equipment were used. The EPNs experienced a pressure differential when going from the pressure chamber to ambient at the orifice outlet. This is similar to the pressure drop that would be experienced by EPNs when passing through the exit orifice of a hydraulic nozzle to the atmosphere.

Images of *H. bacteriophora* after experiencing pressure differentials of (A) 1283 kPa and (B) 10,690 kPa are shown in Fig. 1. At high-pressure differentials, the EPNs broke into pieces such that the internal matter of the EPNs leaked out leaving a transparent sheath (Fig. 1B). At this point, the actual mechanism for breakage due to the pressure differential is not clear.

The mean EPN suspension concentrations initially and after treatment (averaged over pressure differential treatments, including both living and total dead EPNs) for each EPN species are reported in Table 1. For each EPN species, the mean initial concentration was significantly greater than the mean concentration after treatment. It was observed that a small portion of the suspension remained in the French press chamber after each pressure differential treatment. Because of this, samples of *H. megidis* nematodes left in the chamber after the pressure differential treatments of 2138 and 6414 kPa were collected and the relative viability was assessed. The high concentration of living EPNs left

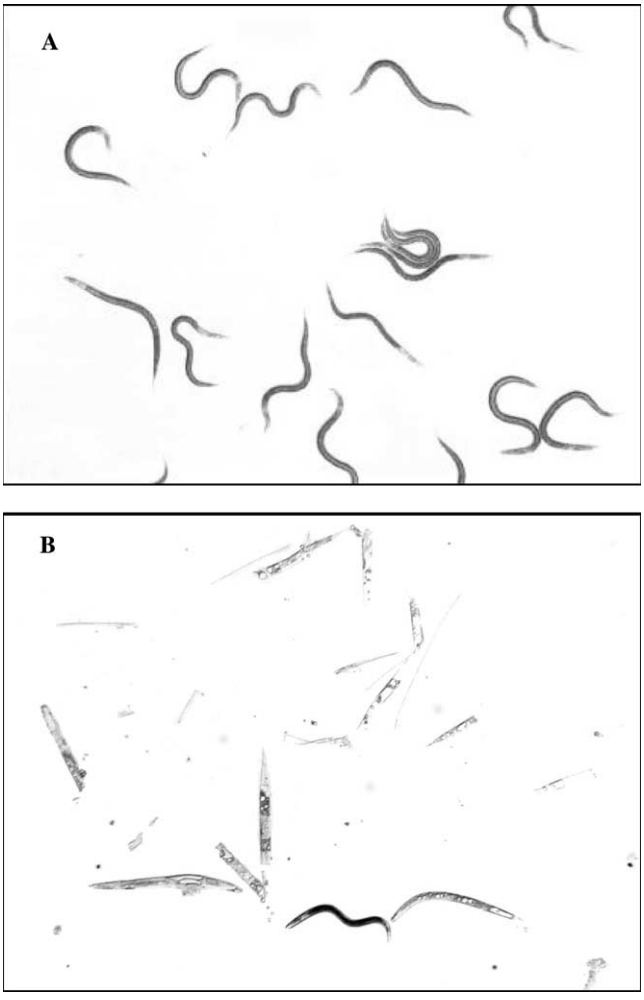

Fig. 1. *Heterorhabditis bacteriophora* after pressure differential treatments of (A) 1283 kPa and (B) 10,690 kPa.

Table 1
Comparison of initial and after pressure differential treatment concentrations of fresh *S. carpocapsae*, *H. bacteriophora*, and *H. megidis*

EPN species	Initial concentration[a,b,c] (EPN/ml)	After treatment concentration (EPN/ml)
S. carpocapsae	12,400 ± 1580 a A	3600 ± 190 b A
H. bacteriophora	14,900 ± 1310 a A	11,600 ± 550 b B
H. megidis	17,200 ± 1430 a A	5200 ± 275 b C

[a] Means followed by ±SE (for initial, $n = 8$ for *S. carpocapsae* and *H. bacteriophora*, and $n = 4$ for *H. megidis*; for after treatment, $n = 90$ for *S. carpocapsae*, $n = 60$ for *H. bacteriophora* and *H. megidis*).

[b] Means in same row followed by the same lowercase letter do not differ significantly according to the Student's t test, unequal variances, $P < 0.05$.

[c] Means in same column followed by the same uppercase letter do not differ significantly according to the Student's t test, equal variances, $P < 0.05$.

in the chamber (25,500 EPNs/ml for 2138 kPa and 30,100 EPNs/ml for 6414 kPa) was nearly two times the initial concentrations, suggesting that clogging occurred upstream from the release valve in the chamber. The relative viabilities of the EPNs left in the chamber were 98.5% for 2138 kPa and 91.8% for 6414 kPa. In both cases, the relative viability was well above the corresponding pressure differential treated relative viability (66.6% for 2138 kPa and 26.4% for 6414 kPa). For 2138 kPa, the effect on relative viability of EPNs left in the chamber was negligible compared to the damage observed from the pressure differential treated EPNs. This suggests that the damage observed for lower pressure differential treatments (<2000 kPa) can be attributed solely to the pressure differential. For 6414 kPa, although the relative viability of EPNs left in the chamber was much higher than the pressure differential treated EPNs, more damage was observed compared to EPNs left in the chamber at 2138 kPa. This suggests that at high pressures some damage to the EPNs may have occurred as the suspension was forced through the release valve. Separation of the damage from passage through the release valve and that due solely to the pressure differential at the exit orifice was impossible to determine in this study. Thus, for higher pressure differential treatments (>3000 kPa), it should be noted that some of the damage may be occurring due to flow effects near the release valve.

Further, shear and extensional stresses are present during the flow through the valve and sample outlet tube and their effects also cannot be separated. Since the flow is extremely slow (one droplet per 5 s), it is reasonable to neglect the hydrodynamic effects of the flow on the overall damage to the EPNs.

The relative viability of each EPN species after experiencing pressure differential treatments is displayed in Fig. 2. The trend is similar for all three EPN species; a high level of relative viability (greater than 80%) for pressure differentials less than or equal to 1710 kPa, then a decrease in relative viability, and finally a lower plateau of relative viability (approximately 55% for *S. carpocapsae* and 20% for *H. bacteriophora* and *H. megidis*) for pressure differentials greater than or equal to 3848 kPa. From the ANOVA, differences in relative viability among EPN species ($F = 48.42$, df = 2, $P < 0.001$), among pressure differential treatments ($F = 65.30$, df = 10, $P < 0.001$), and for a treatment interaction ($F = 3.95$, df = 20, $P < 0.01$) were all statistically significant.

Comparison of relative viability treatment means provides further insight. Overall, the effect of pressure differentials on EPN damage was more pronounced for *H. megidis* compared to the other two EPN species. For pressure differentials less than or equal to 1283 kPa, the mean treatment differences were insignificant for the three species. However, for intermediate pressure differentials between 1283 and 2566 kPa, *H. megidis* had significantly lower relative viability compared to *S. carpocapsae* and *H. bacteriophora*. In particular, by approximately 2000 kPa the relative viability of *H. megidis* had decreased to 67% compared to approximately 90% for *S. carpocapsae* and *H. bacteriophora*. *S. carpocapsae* and *H. bacteriophora* relative viabilities were not significantly different for intermediate pressure differentials. The greatest difference in treatment means between all three EPN species was observed for pressure differentials between 2566 and 3421 kPa. At pressure differentials greater than 3421 kPa, *H. bacteriophora* and *H. megidis* had relative viabilities significantly less than *S. carpocapsae* (approximately 20% compared to 55%), and were not significantly different from each other.

Based on these results, it is recommended that to maintain EPN viability greater than 85%, operating pressures less than 1380 kPa (200 psi) for *H. megidis* and less than 2000 kPa (290 psi) for *S. carpocapsae* and *H.*

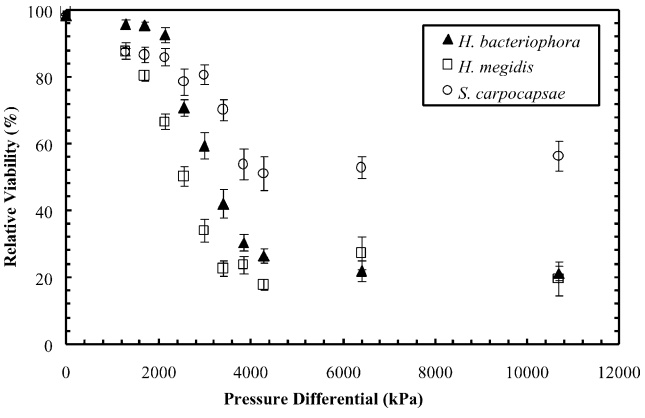

Fig. 2. Relative viability of fresh *S. carpocapsae*, *H. bacteriophora*, and *H. megidis* after the pressure differential treatments. Error bars represent standard error of the mean ($n = 9$ for *S. carpocapsae*; $n = 6$ for *H. bacteriophora* and *H. megidis*).

bacteriophora be used. For soil applied chemical pesticides and herbicides, operating pressures are typically less than 700 kPa. Most, if not all, EPN species can be safely applied to the soil at these operating pressures. However, for foliar coverage of chemical insecticides and fungicides, operating pressures reaching 2000 kPa are common. Thus, for foliar application, EPN species must be considered when deciding on operating conditions in order to maintain high EPN viability. Some EPN species, like *H. megidis*, will experience a significant reduction in viability at these operating pressures, which will result in a reduction of the potential efficacy. Further information on the effect of pressure differentials to other EPN species not evaluated in this study is necessary to develop complete guidelines for optimal operating conditions.

An explanation for the differences in response of the EPN species to pressure differential is unclear, but there may be several underlying factors involved related to EPN morphology. First, the ultrastructure properties of the nematode cuticle may be a factor. *S. carpocapsae* has a proportionally greater striated layer in the cuticle of infective juveniles (IJs) compared to several other *Steinernema* spp. (Patel and Wright, 1998). Kondo and Ishibashi (1989) indicate that the importance of the striated layer is to provide structural support to the nematode cuticle. The structural integrity of the cuticle may be stronger for *S. carpocapsae* compared to *H. bacteriophora* and *H. megidis*, which may provide an explanation for the lower damage observed by this species in this study after pressure differential treatments. However, there is no information currently available on the cuticle ultrastructure of *H. bacteriophora* and *H. megidis* to support this comparison. Another factor may be the size of the EPNs. The average lengths and widths of the infective juvenile *S. carpocapsae* are 558 μm × 25 μm, of *H. bacteriophora* 588 μm × 23 μm, and of *H. megidis* 768 μm × 29 μm (Poinar, 1990). *Heterorhabditis megidis* is considerably longer than the other two species, which may have contributed to the lower relative viabilities observed at pressure differentials between 1283 and 2566 kPa.

No significant mean differences ($P > 0.13$; except at 10,690 kPa, $P = 0.04$) were detected between relative viabilities that were measured the same day as the pressure treatments (0 day) and after 1 week at room temperature (7 day) at each pressure differential level for *H. bacteriophora*. Similar results were observed for *S. carpocapsae* and *H. megidis*; however, there were some significant mean differences detected between the 0-day and 7-day relative viabilities for both *S. carpocapsae* and *H. megidis*. For *S. carpocapsae*, at pressure differential levels greater than or equal to 3421 kPa, the 7-day relative viability remained at approximately 95%, about 30% higher than the corresponding 0-day values. An explanation for the high 7-day relative viabilities could not be determined. For *H. megidis*, at pressure differential levels greater than or equal to 2566 kPa, some relative viabilities for 7 day were significantly lower than 0 day (2566 kPa, $P = 0.01$; 2993 kPa, $P = 0.015$; 3421 kPa, $P = 5 \times 10^{-5}$; 3848 kPa, $P = 6 \times 10^{-4}$; 4276 kPa, $P = 0.01$; 6414 kPa, $P = 0.003$). However, the survival results at high-pressure differentials were not consistent for *H. megidis* and a conclusion could not be inferred. Overall, the survival study suggests that EPNs that were able to survive the pressure differential treatment were able to survive for at least 1 week afterwards.

The mean infectivities of pressure treated EPNs after 72 h were 43.2% (SE ± 2.0%) for *S. carpocapsae* (one-on-one bioassay), 61.7% (SE ± 1.7%) for *H. bacteriophora* (one-on-five bioassay), 81.2% (SE ± 0.9%) for *H. megidis* (one-on-five bioassay), and 43.1% (SE ± 3.0%) for *H. megidis* (one-on-one bioassay). It is not appropriate to compare the levels of infectivity of the different EPN species because of intrinsic differences in the biology and foraging behavior of each EPN species (Grewal and Georgis, 1998). The one-on-one bioassay provides consistent results of near 50% mortality of *G. mellonella* within 48 h for *S. carpocapsae* (Georgis, 1990), as was observed in this study. Less consistent results with the one-on-one bioassay have been achieved with *H. bacteriophora* (Grewal, unpublished data), which is why a one-on-five bioassay was used for *H. bacteriophora* in this study. Less is known about the infectivity rates of *H. megidis*, so both the one-on-one bioassay and one-on-five bioassay were conducted. In multiple EPN assays, individuals are lost in the larger population effects of multiple invasion into a host, and explains the higher infectivity of *H. bacteriophora* and *H. megidis* observed in the current study for the one-on-five bioassay.

There was insufficient evidence to conclude that the EPN infectivity varied with an increase in pressure differential treatment after 72 h for *S. carpocapsae* ($F = 0.44$, $df = 1$, $P = 0.5$), *H. bacteriophora* ($F = 0.56$, $df = 1$, $P = 0.5$), or *H. megidis* ($F = 0.75$, $df = 1$, $P = 0.4$). The EPNs that were selected for the infectivity tests were all living which narrowed the population pool, particularly for the EPNs that experienced a high-pressure differential treatment. These results suggest that the EPNs that survived the pressure differential were not damaged and were able to maintain infectivity rates equivalent to those EPNs that had not been pressure treated.

3.2. Effect of pressure differentials on aged EPNs

The relative viabilities of aged and, for comparison, fresh (A) *H. bacteriophora* and (B) *H. megidis* after experiencing pressure differential treatments are displayed in Fig. 3. Overall, the relative viabilities of aged *H. bacteriophora* and *H. megidis* were not significantly lower than fresh nematodes after experiencing pressure

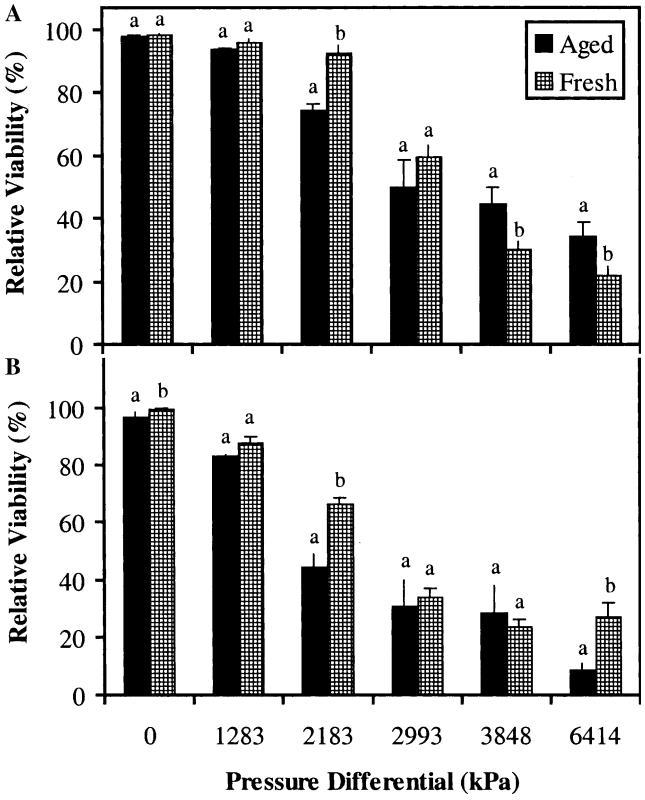

Fig. 3. Relative viability of aged (3 weeks) and fresh (A) *H. bacteriophora* and (B) *H. megidis* after the pressure differential treatments. Error bars represent standard error of the mean ($n = 3$ for aged EPNs; $n = 6$ for fresh EPNs); at a given pressure differential, bars with differing letters above them were found significantly different according to the Student's t test.

differential treatments ($P = 0.48$ and $P = 0.35$, respectively). However, there were a few individual treatment differences. The relative viability of aged *H. bacteriophora* was significantly less than fresh EPNs at the pressure differential of 2183 kPa ($P = 8 \times 10^{-4}$), and significantly greater at 3848 kPa ($P = 0.01$), and 6414 kPa ($P = 0.03$). For *H. megidis*, the relative viabilities of aged nematodes were significantly less than fresh at the pressure differentials of 2183 kPa ($P = 6 \times 10^{-4}$) and 6414 kPa ($P = 0.02$). However, in general, the results from this study indicate that nematode age is not an important factor with respect to the effects of a pressure differential.

3.3. Effect of a static pressure on EPNs

No significant difference in relative viability was detected between *H. bacteriophora* nematodes treated with the static pressure for 0 (control), 5, 10, and 30 min (average relative viability = 99.7%, SE = 0.09%, $P > 0.5$). The static pressure test was conducted at 3421 kPa because this was the mid-range of the pressure differential treatments studied. This result supports the assumption that exposure to a static pressure in the chamber was not a contributing factor to the damage observed. However, it is important to note that there exists a critical static pressure above which an organism can no longer maintain its turgidity with respect to the external pressure being applied and will burst, but this static pressure is anticipated to be much higher than normal operating conditions in a sprayer.

Dutky (1974) reported that *Steinernema* spp. can withstand pressures up to 6900 kPa, but it is not clear whether this assessment was under static or dynamic pressure conditions or to what extent, if any, the EPNs were damaged. In the current study, at a static pressure of 3421 kPa, no damage of *H. bacteriophora* was observed, which was similar to results found by Dutky (1974) for *Steinernema* spp. However, in the current study, at pressure differentials greater than approximately 4000 kPa, only one half of *S. carpocapsae* and one fifth of *H. bacteriophora* and *H. megidis* survived. This reduction in relative viability at pressure differentials up to 6900 kPa contradicts Dutky (1974). The mechanical response of a body being acted on by a static pressure compared to a pressure differential is different. When a body is completely immersed in a container at high pressure, the static pressure acts equally on all sides of the body so that there should not be any deformation due to the static pressure. When a body experiences a pressure differential, the pressure no longer acts equally on all sides, which causes the body to deform. It is this forced body deformation which causes the organism damage. Thus, an organism can withstand a higher static pressure than a pressure differential, as was observed in this study.

3.4. Impact

Ideally, to maintain efficacy against an insect pest, the proportion of viable EPNs being delivered at a given application rate needs to be as high as possible. At present, there are no sound research-based guidelines on nozzle orifice size and operating conditions for field application of different EPN species. Present recommendations, based on *S. carpocapsae*, are for operating pressures less than 2000 kPa, which agrees with results from our study for *S. carpocapsae*. However, greater reductions in relative viability were observed for *Heterorhabditis* spp., in particular *H. megidis*, indicating that EPN species is an important factor to consider when defining spray operation conditions.

Results from this study indicate that the magnitude of the pressure differential has an effect on the relative viability of the EPNs, and the effect is species dependent. Results also suggest that if the EPNs can survive the pressure drop when passing through the nozzle into the atmosphere, then they have the potential to be efficacious in the field. However, as the pressure differential increases, the number of living EPNs with this potential

decreases, reducing potential efficacy. Ideally, the nozzle orifice size must be large enough to accommodate the EPNs, and the operating pressure must be high enough that it provides sufficient velocity energy for the droplets to reach the target, but not too high that the number of viable EPNs is reduced significantly. To maintain viability above 85%, we recommend that operating pressures less than 1380 kPa be used for *H. megidis*, and less than 2000 kPa for *H. bacteriophora* and *S. carpocapsae*.

This study provides basic information on the effect of one spray parameter, operating pressure, on the viability and infectivity of EPNs during the delivery process. Ultimately, research-based guidelines that define all operating conditions for efficient and efficacious delivery of biological pest control agents is essential for increased acceptance and use by growers. Further research is underway to evaluate the effect of hydrodynamic stresses on EPNs during flow through a constriction, such as a hydraulic nozzle or valve opening.

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